

Maertens et al.
Serial No. 09/899,303

Insert the attached drawings for those which were originally filed.

IN THE CLAIMS

Amend the claims as follows.

Cancel claims 49-66, without prejudice.

Add the following claims.

B4
DRAFTING COPIES OF DRAWINGS
67. (new) A recombinant vector comprising a vector sequence, an appropriate prokaryotic, eukaryotic or viral promoter sequence followed by a nucleotide sequence allowing the expression of an HCV single or specifically oligomerized envelope viral protein selected from the group consisting of E1 and/or E2 and/or E1/E2 viral proteins.

68. (new) The recombinant vector according to claim 67, with said nucleotide sequence being characterised further in that it encodes a single HCV E1 protein starting in the region between amino acid positions 1 and 192 and ending in the region between amino acid positions 250 and 400, more particularly ending in the region between positions 250 and 341, even more preferably ending in the region between position 290 and 341.

69. (new) The recombinant vector according to claim 68, with said nucleotide sequence being characterised further in that it encodes a single HCV E1 protein starting in the region between amino acid positions 117 and 192 and ending in the region between amino acid positions 263 and 400, more particularly ending in the region between positions 290 and 326.

✓ 70. (new) The recombinant vector according to claim 68, with said nucleotide sequence being characterised further in that it encodes a single HCV E1 protein bearing a deletion of the first hydrophobic domain between positions 264 to 293, plus or minus 8 amino acids.

71. (new) The recombinant vector according to claim 67, with said nucleotide sequence being characterised further in that it encodes a single HCV E2 protein starting in the region between amino acid positions 290 and 406 and ending in the region between amino acid positions 600 and 820, more particularly starting in the region between positions 322 and 406, even more preferably starting in the region between position 347 and 406 and most preferably starting in the region between positions 364 and 406.

72. (new) The recombinant vector according to claim 71, with said nucleotide sequence being characterised further in that it ends at any of amino acid positions 623, 650, 661, 673, 710, 715, 720, 746 or 809.

73. (new) The recombinant vector according to claim 67, with said nucleotide sequence further comprising operably linked a 5'-terminal ATG codon and a 3'-terminal stop codon.

74. (new) The recombinant vector according to claim 67, with said nucleotide sequence being characterised further in that a factor Xa cleavage site and/or 3 to 10, preferably 6, histidine codons have been added 3'-terminally to the coding region.

75. (new) A recombinant vector comprising any of the sequences as represented in SEQ ID No 3, 5, 7, 9, 11, 13, 21, 23, 25, 27, 29, 31, 35, 37, 39, 41, 43, 45, 47 and 49, or parts thereof.

76. (new) The recombinant vector according to claim 67, further characterised in that at least one of the glycosylation sites present in said E1 or E2 protein has been removed at the nucleic acid level.

77. (new) A vaccine composition comprising a recombinant vector according to claim 67.

78. (new) A method of vaccinating a human comprising administering a vaccine composition of claim 77.

79. (new) A composition comprising a recombinant vector according to claim 67.

80. (new) A host cell transformed with at least one recombinant vector according to claim 67, wherein said vector comprises a nucleotide sequence encoding HCV E1 and/or E2 and/or E1/E2 protein and a regulatory sequence operable in said host cell and capable of regulating expression of said HCV E1 and/or E2 and/or E1/E2 protein.

81. (new) A recombinant E1 and/or E2 and/or E1/E2 protein expressed by a host cell according to claim 80.

82. (new) A recombinant E1 and/or E2 and/or E1/E2 protein according to claim 81, further characterised in that said host cells are mammalian cells.

83. (new) A recombinant E1 and/or E2 and/or E1/E2 protein according to claim 81, further characterised in that said host cells are yeast cells.

84. (new) Method for purifying HCV envelope proteins selected from the group consisting of E1 and/or E2 and/or E1/E2, characterised as comprising at least the following steps:

- growing a host cell as defined in claim 80, transformed with said recombinant vector, in a suitable culture medium,
- expressing said vector sequence under suitable conditions, and,
- lysing said transformed host cells, preferably in the presence of an SH group blocking agent, such as N-ethylmaleimide (NEM),
- recovering said HCV envelope protein by affinity purification by means of for instance lectin-chromatography or immunoaffinity chromatography using anti-E1 and/or anti-E2 specific monoclonal antibodies, with said lectin being preferably lentil-lectin, followed by,
- incubation of the eluate of the previous step with a disulphide bond cleavage agent, such as DTT, preferably also in the presence of an SH group blocking agent, such as NEM or Biotin-NEM, and,
- isolating the HCV single or specifically oligomerised E1 and/or E2 and/or E1/E2 proteins by means of gelfiltration and possibly also by means of an additional Ni²⁺-IMAC chromatography and desalting step.

85. (new) A method for immunizing a human comprising administering a composition of claim 79. 142

86. (new) The composition according to claim 79, further comprising a pharmaceutically acceptable adjuvant.